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EUPLOTES WORCESTERI SP. NOV.: I. STRUCTURE.[1]

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This Euplotes was discovered in February, 1909, in some water from Manila Bay which was brought to the laboratory with the eggs of an Aplysiid. As the eggs decayed an infusion was produced in which the Euplotes multiplied freely. My attention was directed to this form by observing the process of division in a living individual and noting that the new peristome appeared to lie, not on the outer surface lateral or posterior to the old one, but inside the body, dorsal to the old peristome, remaining thus until the two halves of the body drew apart. This observation was so at variance with the processes of division occurring in the Protozoa with which I was then acquainted, that I made considerable effort to verify it. Further study disclosed numerous other features so interesting that it seemed desirable to make a complete study of the structure, division, and conjugation of the species. When this Euplotes was discovered there were at least three other species of hypotrichous infusoria in the culture, several species of other ciliates, together with flagellates and amœbæ. The Euplotes multiplied with far greater freedom than any of the other protozoa. From time to time since then, the growth of the Euplotes has been stimulated by dropping into the jar small pieces of thoroughly cooked fish or Arcas. The cooking was simply to avoid the possibility of adding fresh protozoa and bacteria to the culture. Whenever a proper interval had passed since the previous infusion, a rapid increase of the Euplotes always took place, so that by the third day after starting a fresh infusion the animals could be found by hundreds in any drop taken from the under surface of the bacterial scum covering the water. The cultures usually reached their maximum development two or three days later; a few hours after this stage almost all the animals died. It was then impossible to bring about a multiplication of the animals in this jar by starting a fresh infusion until after the processes of decomposition of the preceding one had been completed, and the water cleared. Nevertheless, a few Euplotes could be found near the surface of the water at any time during this period.

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A blue-green alga (an *Oscillatoria*) grew well on the sides of the jar from the beginning, and probably helped to preserve conditions favorable to the Euplotes. The other protozoa died out gradually until at the end of a year only one other form, a species of Lembus, remained with the Euplotes.

This Euplotes appears to be an undescribed species, very closely related to *E. vannus;* comparisons therefore are closely limited to the two forms. The new species is named in honor of the Hon. Dean C. Worcester, who has been interested in the study of protozoa for many years, and who has vigorously assisted the rapid development of scientific work and education in the Philippines.

The form of the animal is shown in Plate I, figure 1, and Plate II, figures 2, 3, and 4. The ventral surface is always flat, the dorsal surface considerably elevated in normal individuals. The curvature of the dorsal surface varies to a considerable degree in accordance with the amount of food eaten, so that in a fresh, rich infusion the animals are thicker bodied than in an old or poor infusion. In every culture some Euplotes can always be found which are much broader than the normal, oval in outline, and greatly flattened.

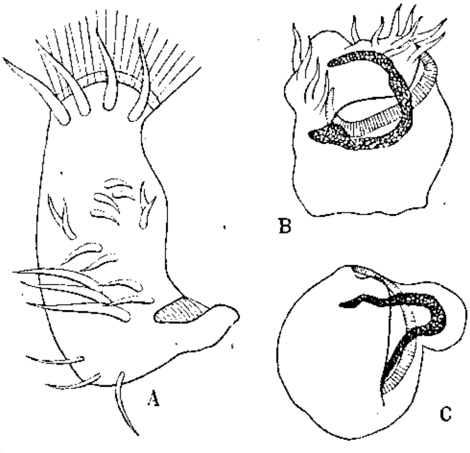

FIG. 1.—Outline sketches of three of the deformed individuals which appear when cultures have passed their prime. Specimen A seems to be the result of an aborted division.

After cultures have passed their prime, greatly malformed individuals are sometimes common. These are always much flattened, the dorsal and ventral surfaces are parallel, and the thickness is sometimes only one-tenth of the width. A great distortion of the form of the body accompanied by changes in the shape and position of the peristome frequently occurs. These abnormal forms probably are the result of the same conditions which presently cause the death of the greater part of the animals in the culture, that is, the accumulation of waste products and toxins of the Euplotes themselves and of the bacteria present.

From Plate I, figure 1, and Plate II, figures 2 and 4, it will be seen that the greater part of the peristome lies upon the ventral surface, in the shape of a shallow depression the median wall of which is vertical, while the lateral wall curves from the bottom of the peristome to the level of the ventral surface. The anterior portion of the peristome passes as a shallow vertical depression across the anterior end of the body to the right side. The inner wall of the peristome turns abruptly to form the ventral edge of this groove, while the lateral edge of the peristome passes over the left edge and then across the anterior end of the body. The anterior portion of the peristome is slightly overhung by the projecting dorsal surface.

A thin lamella projects from the margin of the inner wall, commencing at the right end of the peristome and continuing around the angle of the median wall. The vertical anterior portion of the peristome thus lies between the projecting ledge of the dorsal surface and the marginal lamella. (Plate I, figure 1; Plate II, figure 2.) I have never found this marginal lamella extending far back of the angle of the peristome. Minkiewicz publishes several sketches showing it to extend as far as the mouth in *E. vannus*. In other species of Euplotes, for example *E. harpa,* the marginal lamella is developed to a much greater extent. It is not vibratile, its function being purely passive.

Posteriorly, the lateral edge of the peristome turns toward the mid-line in a graceful curve, which is continued into the posterior wall of the pharynx. The posterior margin of the peristome is deepest next to the mouth, and the lateral wall becomes more and more nearly vertical as it approaches the mouth. The inner margin of the peristome sometimes stops where it joins the outer margin back of the mouth, but usually continues as a low ridge for a little distance past that point. A slight curvature of the inner wall of the peristome occurs below the mouth. (Plate I, figure 1, Plate II, figure 4.) The adoral zone of membranellæ lies close against the outer margin of the peristome, leaving the median portion of the groove free from cilia or other vibratile organs.

The ventral surface curves dorsad rather abruptly back of the five large anal cirri, then again becomes horizontal; the caudal end of the body is thus much thinner than other parts. A low ridge extends along the right margin of the ventral surface. Each of the anal cirri stands in the posterior end of an elongated depression of the surface, separated by ridges which attain a far greater development in other species of the genus.

Mouth and pharynx.—The mouth is situated upon the vertical median wall of the peristome. The curving posterior side of the peristome forms its posterior and part of its dorsal margin.

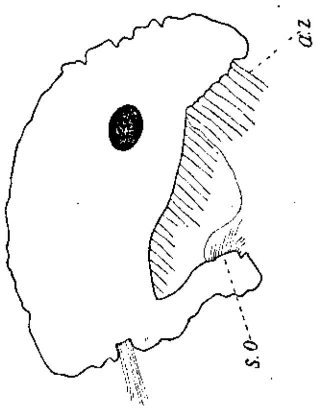

FIG. 2.—A cross section passing through the mouth, the adoral membranellæ, *a. z.*, and the suboral membranellæ, *s. o.*

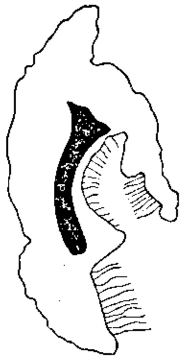

FIG. 3.—Cross section passing through the mouth, the suboral membranellæ, the pharynx and pharyngeal membranellæ, and the adoral zone.

The outline of the mouth, instead of being regularly round or oval, is quite irregular, being about twice as wide along the ventral edge as along the dorsal. The ventral extension of the mouth and pharynx accommodates a group of membranellæ to which I have applied the name *suboral.* (Plate I, figure 1; Plate III, figure 5; text figures 2 and 3.) The bases of the suboral membranellæ lie in a definite oval area, possibly a region where the pellicle is thinned. By examining living specimens it was impossible to determine whether the suboral membranellæ were upon the ventral lip of the mouth, or upon the projection of the anterodorsal wall. The latter seemed to be the case. It was only when sections had been made that the exact position of the suboral membranellæ was demonstrated. Just posterior to the suboral membranellæ is a small elevation of the floor of the pharynx. (Text figures 3 and 6.)

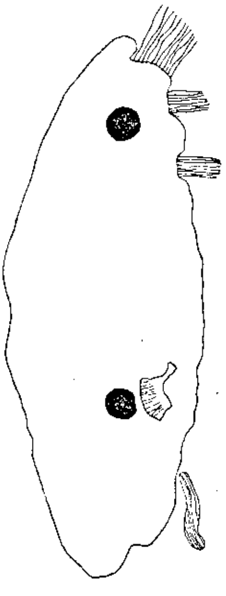

FIG. 4.—A longitudinal section, showing the anteroventral groove of the pharynx.

The pharynx passes to the right and forward, its posterior outline being a continuation of the regular curve of the outer wall of the peristome. The pharynx narrows considerably just inside the mouth, after which the anterior wall approaches the posterior very gradually. The inner end of the pharynx is blunt and comparatively wide. The anteroventral angle of the pharynx forms a deep groove which extends from the corresponding angle of the mouth to past the middle of the pharynx. (Plate III, figure 5; text figure 4.)

The zone of adoral membranellæ continues into the pharynx without a break, although with a gradual reduction in the length and breadth of the individual membranellæ. The membranellæ of this series which lie inside the pharynx may, for convenience, be termed the *pharyngeal* membranellæ. They vary in number; usually there are from twenty to twenty-four, though a few more or less may be found. It will be observed that twenty-four membranellæ are shown in text figure 5, and that this section does not include quite the full length of the pharynx. Although the number of the membranellæ inside of the pharynx is subject to considerable variation, I have never noticed so small a number as 8, which is the number Minkiewicz found in his Black Sea specimens of *E. vannus*. I also find the pharynx to extend considerably farther into the body than is represented by the figures of Minkiewicz. It is often only by very careful examination, even with an immersion lens, that the inner end of the pharynx can accurately be outlined, partly because of the thickness of overlying granular protoplasm, and partly because of the increased delicacy of the vibratile organs in the inner part of the pharynx and of the walls of the pharynx itself.

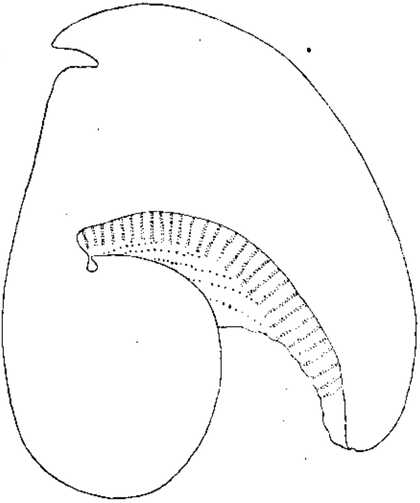

FIG. 5.—A tangential section passing through the dorsal wall of the pharynx. The rows of basal granules of the pharyngeal membranellæ and of the endoral cilia show the arrangement of these organs.

The pharyngeal membranellæ lie on the rounded posterodorsal surface of the pharynx, leaving part of the dorsal surface which is not occupied

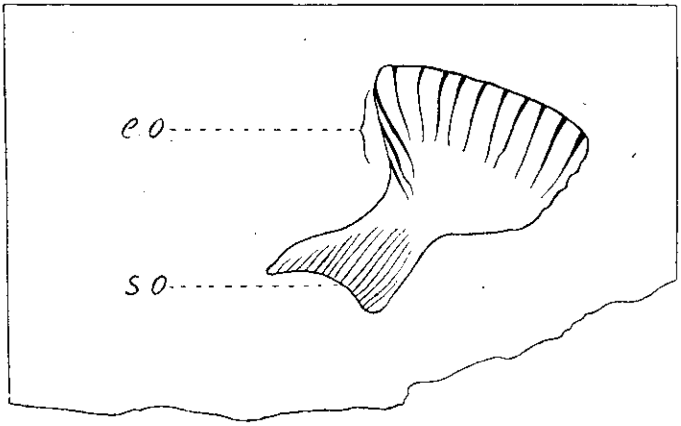

FIG. 6.—Part of a longitudinal section passing just inside the mouth. *s. o.*, suboral membranellæ; *e. o.*, endoral cilia, which in this section are well down on the anterior wall.

by them. In this space lie several oblique rows of fine cilia which probably are homologous with the endoral cilia of other Hypotricha. (See text figures 5 and 6; Plate I, figure 1.) At the oral aperture three or four of these rows (occasionally more) lie side by side, forming a ciliated band which extends from the mouth to the inner end of the pharynx, narrowing slightly as it passes inward. These cilia are the most difficult feature of the ciliation to observe in the living animal, but with favorable specimens and using high powers of magnification the lines of vibrating organs can be seen. However, I could not be sure of the observation nor of the nature of the organs without recourse to sections, which showed the cilia and their arrangement with sufficient clearness. (See text figures 5 and 6.) The ends of the rows of endoral cilia may extend upon the anterior wall of the pharynx. (See text figure 6.)

I have since found the suboral group of membranellæ and the endoral cilia in another unidentified species of Euplotes, and therefore venture to suggest the possibility that these structures may be found in still other species of the genus. The praeoral cilia of *E. harpa* described by Wallengren are different in position and arrangement from both the suboral membranellæ and the endoral cilia of *E. worcesteri*. They may, however, be homologous to the endoral cilia.

The extreme shortness of the pharynx is a noticeable feature in most of the Hypotricha. The organ reaches its greatest development in the Euplotidæ, especially in the genus Euplotes. The pharynx of *Euplotes worcesteri* is longer than that of any other Euplotes known; however, that of *E. vannus* is only slightly shorter. In other species of the genus the length of the pharynx is much less. The forward curvature of the pharynx of *E. worcesteri* and *E. vannus* also is remarkable among Hypotricha.

The adoral and pharyngeal membranellæ and the endoral cilia are in nearly constant motion. In addition to gathering food particles the anterior adoral membranellæ appear to be the principal natatory organs of the animal. The suboral membranellæ, on the contrary, are apt to be found at rest except when food is being swallowed. These membranellæ appear to be triangular. The small area covered by them is oval in outline, its axis obliquely inclined to the ventral edge of the oval aperture. The tips of the outermost membranellæ project slightly from the mouth, and just clear the tips of the adoral membranellæ. Direct observation, many times repeated, has proved that food particles which arrive at the mouth are seized between the adoral membranellæ above and the suboral below, and forced into the pharynx.

That the thrusting power of the two sets of membranellæ is considerable was shown one day when I observed a Euplotes which had captured and was trying to swallow a small Lembus rather more than one-half as long as itself. When first seen the Euplotes had the anterior end of the Lembus in its pharynx. The Lembus was alive and struggling; several times it was forced along the pharynx and its tip entered the protoplasm of the Euplotes; once nearly one-half its body was in the endosarc of its captor. However, each time it forced its way back, and finally escaped altogether from the Euplotes and moved away giving no evidence of having received any harm. On another occasion a Euplotes was found in the act of swallowing an amœba of elongated form. A little less than one-half of the body of the amœba then lay in the pharynx, completely filling about two-thirds of this organ, and blocking the membranellæ so that none of them could act. As a result, after a few efforts the amœba reversed the direction of its motion and crawled out of the pharynx, across the adoral zone, and away. I have frequently observed that small protozoa and bacteria are thrust into the mouth between the adoral and suboral membranellæ and then are pushed along by the pharyngeal membranellæ and cilia; currents of water, conveying food particles from the mouth to the tip of the pharynx, do not appear to exist. A similar conclusion was recently reached by Schäffer regarding the process of swallowing in Stentor.

The function of the endoral cilia is more difficult to understand than that of the suboral membranellæ, because direct observations are almost impossible. Large food bodies which reach the mouth are grasped easily between the two sets of membranellæ. In the case of small particles, like bacteria, the process may be different. Only those which reach the mouth at the level of the tips of the adoral membranellæ will be seized in the manner described. Those which lie in a lower, i. e. more ventral, level will pass over the posterior wall of the peristome with the currents of water. Those which arrive at the dorsal part of the oral aperture will be brought within reach of the endoral cilia and pushed along to the tip of the pharynx. If the Euplotes lies with its dorsal side up, the particles captured by the endoral cilia may fall out of their reach to the ventral side of the pharynx, but there they will be pushed along by the tips of the pharyngeal membranellæ. However, if the Euplotes is swimming with the ventral side up as is frequently the case, the particles captured by the endoral cilia will be thrust along by them until the inner tip of the pharynx is reached. In addition, the inclined position of the pharyngeal membranellæ is such that, with the animal in this position, any particles which escape from them will drop directly upon the endoral cilia and will also be passed down the pharynx. The pharyngeal apparatus of Euplotes is, therefore, a remarkably efficient one.

On the other hand, if we view the adoral zone of membranellæ as a mechanical food conveyor alone, its efficiency is very low, for the currents produced by it are so strong that nearly every particle drawn into the peristome is thrown out again over the posterior border at a high speed.

A very low percentage of particles is thrown into the angle where the mouth is located and large numbers of these eventually are lost. However, the adoral zone is more than a food conveyor, it is also the principal organ of locomotion; a balance of functions exists such that an increase of efficiency in either direction without other modifications would be fatal.

This Euplotes appears to be selective in its feeding, if tests made with carmine are of any value, for I have seen but one specimen which swallowed any carmine granules, and that one took only a few. While bacteria were being swallowed in large numbers, the carmine grains were swept out of the peristome as if the animal were unconscious of their existence. The experiment has been repeated several times and some hundreds of Euplotes observed. It is noticeable also, that when several kinds of food are present in an infusion one kind appears to be preferred, as is proved by the nature of the food masses in the bodies of the infusorians.

Minute food particles are collected in the form of a dense ball at the inner end of the pharynx before being swallowed, as in many other infusorians. Room for this is provided, not in the pharynx itself, but in an evagination of the anterior side, projecting into the endosarc, and increasing in size as the food mass grows. Text figure 5 shows an early stage of the evagination, and a later stage is represented in Plate V, figure 10. When the ball is finally ingested no visible layer of water accompanies it.

Membranellæ.—The number of adoral membranellæ varies between 45 and 70. The anterior ones are strikingly long. (Plate I, figure 1.) Passing toward the mouth, a gradual reduction in length occurs so that near and at the mouth they are able to swing freely within the edges of that opening.

Minkiewicz describes the adoral membranellæ of *E. vannus* as triangular, and Wallengren represents those of *E. harpa* as of a similar shape. After repeated examination I am convinced that the shape of the expanded membranella of *E. worcesteri* is nearly rectangular, about as shown in Plate I, figure 1. However, some are longer on the outer than the inner side, while very frequently during action the membranellæ assume a triangular shape by the inner side being drawn toward the outer. The membranellæ of this genus are extremely flexible organs, showing but little modification from the condition of free cilia.

The membranellæ of this species, even in the living condition, do not appear to be homogeneous. As soon as the motion of these organs slows in a captured specimen, each membranella appears to consist of a double row of delicate cilia, closely set, and moving exactly together but without any visible uniting substance.

After fixation and staining, no matter what the method, the appearance of separate cilia is even more definite. Even where the membranellæ appear to be changed only slightly by the fixation, the cilia of which they are composed are all perfectly distinct and separate. There are no places where a few appear to be fused in a plate, lying regularly and parallel as one would expect to find if the membranellæ were definite protoplasmic plates. Each cilium can clearly be traced from basal granule to tip, as a separate and distinct unit. The individuality of the cilia is well shown by sections cutting the membranellæ above their bases.

It may be that during life there exists a film of protoplasm joining the cilia, too delicate to be seen, and destroyed by the slightest disturbance of normal conditions. I have seen the separate cilia of the membranellæ so many times in the living animal where there seemed to be very little disturbance of conditions, that I am inclined to believe that the membranellæ of this form are composed of distinct cilia with movements so perfectly coördinated that they act and ordinarily appear as a single and delicate blade. Möbius also came to the same conclusion.

The view held by the majority of zoölogists is that the membranellæ are formed by the fusion of cilia. This Euplotes shows the individual cilia better, with less fusion, than the majority of other forms which have been studied. Instead of arguing from the appearances produced by certain reagents, one or two structural characters, and the general probabilities of development, we here are able to see the units of which the organ is composed in the living specimen. This is true of both adoral and suboral membranellæ.

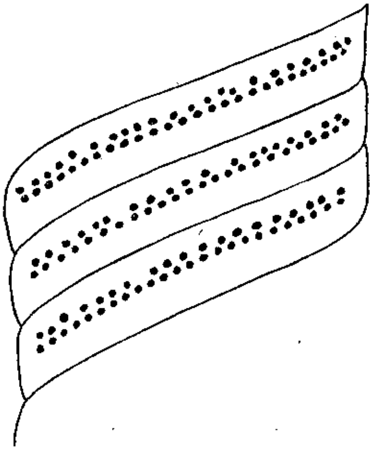

FIG. 7.—Basal granules of three adoral membranellæ and the outlines of the grooves about their bases.

Each membranella of the adoral zone occupies the middle of a shallow groove crossing the zone. The bases of the cilia of the membranellæ are united by a slightly elevated ridge of the ectosarc. The basal ridge stains more deeply than the surrounding protoplasm. At the base of each membranella lies an irregularly double row of large basal granules, a single granule for each cilium.

Cirri.—The arrangement and location of the cirri need no special description, variation from that shown in Plate I, figure 1, being infrequent except for one group. The small cirri shown on the right posterior margin are frequently altogether absent, and when present they may be either one, two, or three in number.

The feature of most interest in regard to the cirri is the ease with which the individual cilia of which they are composed may be demonstrated. After the Euplotes have been confined for only a few minutes under a cover-glass, the tips of the cirri become frayed, and a little later the entire cirrus has changed to a brush of strong cilia which may either beat as a unit, or each cilium may move independently.

All reagents cause the cirri to break more or less into their ciliary elements, while a change in the density of the water will cause them to brush instantly and most markedly of all, though the animal may still be alive. Each of these cilia is connected with a basal granule. (Text figure 8; Plate III, figure 8.) The basal granules lie in a plate of specially dense and stainable, nongranular ectosarc. (Plate III, figure 8.)

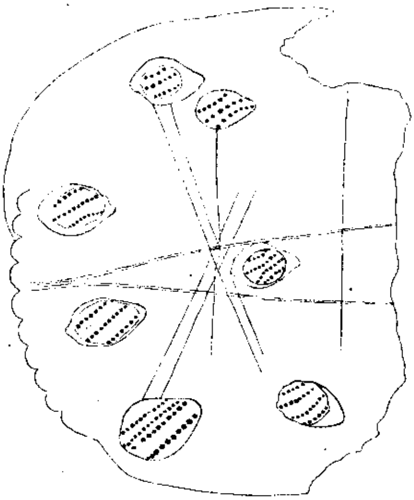

FIG. 8.—Camera lucida sketch of a tangential section of the ventral surface, showing the rows of basal granules and some of the contractile fibrils of the cirri.

On examining tangential sections of the ventral surface of the body, it was found that the basal granules of each cirrus are arranged in several parallel rows (text figure 8), the rows being slightly oblique to the axis of the body. That the cirri are composed of bundles of fused cilia has been believed for many years. This last observation indicates, in addition, that each cirrus has developed, not from a single row, but from several of the rows of cilia which we may imagine to have covered the body of the ancestral form. It has been difficult to reconcile this theory with the observations of Stein and Sterki that the new cirri of Stylonichia arise from portions of a delicate undulating membrane. My own observations of the development of cirri in *Euplotes worcesteri* lead me to believe that this objection is not serious. (See Part II.) In addition Wallengren observed that in *Euplotes harpa* and Stylonichia the rudiments of the cirri appear separately.

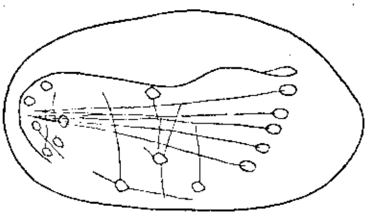

FIG. 9.—Contractile fibrils of the cirri, drawn from an unstained specimen fixed in corrosive-formol-acetic, followed by alcohol.

In connection with the bases of the cirri are certain endoplasmic fibers. (Plate II, figure 4; text figure 9; Plate III, figure 8.) A fiber passes forward from the base of each anal cirrus, the five fibers gradually converging until they end close to each other at the anterior extremity of the ventral surface. Occasionally they seem to unite as described by Maupas, but I do not find this condition so frequently as the other. These fine fibrils are much larger and more readily seen than the fibrils of the other cirri. I have never seen in one specimen so many fibrils as Prowazek shows in his figures of *E. harpa.* The fibrils of the anal cirri are seen easily in the living animals, and they become quite conspicuous after staining with aceto-carmine. They are not as easily seen in sections, because of the difficulty of cutting sections exactly parallel with their course.

Plate II, figure 4, represents the fibrils seen in a living animal; text figure 9 shows others seen after fixation, but without staining. Plate III, figure 8, is a drawing of a section stained with iron-hæmatoxylin.

From these figures it will be seen that the fibrils could not be traced from the base of every cirrus, and that different arrangements were found in different specimens. In general it may be stated that the fibrils seemed to be most developed in connection with the strongest or most used cirri.

The fibrils lie just inside the ectosarc, or it might be better to say in the inner layer of ectosarc. Each ends in the basal plate of dense protoplasm under the cirrus. They appear to be round in cross-section.

It also will be noticed from the figures that more than one fibril is connected to several of the frontal and abdominal cirri. I have never seen more than the single fibril for each anal cirrus. If the fibrils are contractile, as seems altogether probable, they are developed around the bases of the cirri in such directions as to assist in producing the ordinary motions. As the anal cirri have only a single strong motion, a vigorous kick directed backward, each needs but a single strong fibril. The movements of the other cirri are more varied and consequently the fibrils are more numerous. It may be that such fibrils are connected with all the cirri, but only a few are visible in any specimen; or it is possible that the ordinary contractility of the ectosarc is sufficient to produce the movements of the smaller cirri and that no specialized fibrils exist.

In this connection the fact may be mentioned that the two left marginal cirri (back of the peristome) do not have the striking or kicking motion of the others, but lash rapidly and forcibly with a spiral motion.

Engelmann noticed that a very fine plasma-thread arises from each marginal cirrus of Stylonichia, which can be followed close to the ventral surface nearly to the mid-line of the body. Maupas repeated this observation and also discovered the fibrils of the five anal cirri of Euplotes. The anal cirri of Stylonichia possess similar fibrils. Engelmann suggested that the fibrils were nervous in function, radiating from a common nerve center, but Bütschli believed them to be similar to the intracellular fibrils of ciliated metazoön cells, and probably contractile; this is much the more reasonable view. Every detail of arrangement and structure indicates that the fibrils are, principally at least, contractile in function. A relation with the myonemes of other Ciliata suggests itself immediately; and, if each cirrus represents the highly developed and specialized remnant of several rows of cilia, it seems possible that some of the myonemes of these rows may have remained and developed accordingly. The objection to this theory is twofold: first, that many of the fibrils run in directions which bear no relation to the hypothetical original ciliary rows; and, second, that while each cirrus has several rows of basal granules, the fibrils do not correspond to these rows.

Sensory bristles.—The Hypotricha are commonly described as having numerous immotile bristles on the dorsal surface, which probably are the modified vestiges of a once close ciliation (see Bütschli, Döflein, et al.). A sensory function is ascribed to them, and the name of sensory bristles will be adopted here without questioning the correctness of its application.

I find that *Euplotes worcesteri* possesses not only the usual dorsal bristles, but also several rows and definitely arranged groups of ventral bristles. The bristles of this Euplotes are extremely short and transparent, rendering direct observation most difficult; but as the base of each bristle is usually surrounded with refringent ectosarcal granules, their positions can easily be seen. Plate II, figure 3, is drawn from a specimen in which the bristles were unusually clear. The tips were bluntly pointed, while the bases were expanded. The shape is decidedly different from that of the bristles heretofore described in various Hypotricha by other observers, and from the bristles of other species of Euplotidæ which I myself have observed. The bristles of the ventral surface are even smaller and more difficult to observe than the dorsal ones. I find no record of any previous observation of these ventral sensory bristles, although Stein saw the granules which surround their bases along the sides of the adoral zone of membranellæ in *Euplotes charon* O. F. M. These observations have been repeated many times, and careful examination has been made on numerous occasions to determine that a bristle stood at the center of each group of granules. As the granules are so easily seen, and the bristles are distinguished only with difficulty, there was manifestly some danger of assuming that each group of granules necessarily indicated the position of a bristle. The bristles of the dorsal surface usually lie in eight longitudinal rows, although six, nine, or ten rows are found. It happens that Plate II, figure 3, is drawn from the only specimen I have ever seen having six rows.

Upon the ventral surface the bristles are always arranged as follows: First, there are several bristles about the base of each cirrus; usually four, occasionally six, at the bases of the large cirri, and two or three beside the smaller ones. (Plate II, figure 4.) Second, there is a row of bristles on each side of the adoral zone of membranellæ; with few exceptions a bristle stands opposite either end of each membranella. Third, a short row of larger bristles lies along the posterior half of the left margin. These bristles are of about the same size and are spaced about the same as the bristles of the dorsal surface. The arrangement of these sensory bristles of the ventral surface gives further support to the view that the bristles are modified cilia. The resemblance between the row of bristles on the inner side of the adoral zone to what some authors call the paroral or preoral cilia of forms such as Stylonichia and Urostyla is obvious. The outer row of bristles evidently represents a marginal row of cilia.

Pellicle.—The body is covered by a thin tough pellicle which is usually very difficult to distinguish from the outer layer of protoplasm. Some specimens, after treatment with a modification of the Golgi method, show a quite regular network of wrinkles in the pellicle. On another occasion an accident in focusing caused the breaking and partial crushing of a number of stained specimens. The pellicle was then easily distinguished where it projected beyond the protoplasm at broken edges, and also where it had shrunk and wrinkled on the surface of the body as soon as the slight tension to which it had been subjected was relieved. The pellicle is extremely clear and transparent, and shows no structure.

The cirri project through openings in the pellicle which are a little larger than the bases of those organs, leaving a narrow space around the base of each cirrus where the protoplasm is unprotected. In one of my cultures great numbers of small amœbæ appeared, and I then observed several Euplotes which were attacked by the amœbæ, the latter thrusting part of the body into the Euplotes through the unprotected space at the base of a cirrus, while half or more of the body remained outside.

Ectosarc (Plate III).—The ectosarc forms a very definite layer of considerable thickness, which can be distinguished easily in both living and stained specimens. The marked feature of the ectosarc is a single layer of large alveoli filled with clear, homogeneous, and apparently fluid substance. These alveoli, viewed from the surface, give the ectosarc very much the appearance of being composed of cells. (Plate III, figure 7.) The walls separating the alveoli are usually very thin, but may contain smaller alveoli of similar nature. Their depth is in most cases almost the thickness of the ectosarc, although it is not uncommon to find two layers of smaller alveoli. The contents of the alveoli stain with Lyons blue, while the walls do not; Lyons blue used in combination with acetic-carmine gives a very sharp picture of the alveolar structure of the ectosarc. The contents of the alveoli do not shrink, nor stain with iron-hæmatoxylin. The alveoli and their contents are evidently of an entirely different nature from the ectosarc vacuoles and granules described by Metcalf although they have much the same appearance. Between the ends of the alveoli and the pellicle is a thin layer of protoplasm, which may contain other very fine alveoli.

The radiating granules about the bases of the sensory bristles have been mentioned. There are several references in the literature to these structures, which appear to be peculiar to the Euplotidæ, and the questions of their nature and exact location are still unsettled. The granules have been observed by Dujardin, Claparede and Lachmann, Stein, Rees, Stokes, and recently by Minkiewicz. Rees suggested that they might be vacuoles, possibly contractile. Bütschli considered that they were more likely to be pellicular formations. Minkiewicz denies that they can be pellicular, or vacuoles; his statement is that they are "probably" crystalline, and "probably" lie between the ectoplasm and endoplasm.

The granules are extremely variable in size, although those of the ventral surface (excepting those of the lateral row) are always much smaller than those of the dorsal. The granules along the sides of the adoral zone are smallest of all.

The granules are always longer than broad, sometimes three or four times so; colorless, refringent, and crystalline in their appearance. No angles are visible, all sides and the ends apparently being smooth and rounded. The largest granules of the dorsal surface may be 1 μ by 2 μ. More commonly the length is three or four times the width. The smallest ones are not more than one-quarter the size of the larger. The granules of the ventral surface, except those of the lateral row, are rarely more than 1 μ in length, and usually about ½ μ.

The granules always lie around the bases of the sensory bristles, on both the upper and lower surfaces, arranged radially with the base of the bristle as a center. The central ends of the granules are also directed toward the surface of the body, giving a conical form to the group. In many instances the pellicle is actually elevated a trifle over the group of granules, which fact has caused Stokes to interpret them as appearances due to star-shaped elevations of the surface instead of real bodies.

The number of granules in a group varies greatly, but the largest groups are always found on the dorsal surface, while the smallest are seen in the rows beside the adoral zone. In the former there are frequently as many as a dozen granules, while the latter rarely contain more than four very small ones, and frequently only one or two. There is surprisingly little irregularity in the position of these groups of granules. Very rarely one observes a few irregular groups in the center of the ventral surface, but nowhere else.

The granules lie in the outer layer of ectosarc, immediately beneath the pellicle. They are not pellicular structures, as Bütschli considered them, neither do they lie between the ectosarc and endosarc. The presence of the large ectosarc alveoli makes it possible to determine the position of the granules by focusing in either living or stained material, and I have also found a few in sections.

The suggestion of Rees that the groups of granules are vacuoles, possibly contractile, has nothing to commend itself to further consideration.

The granules are not dissolved by strong or dilute acetic, hydrochloric, or nitric acids, nor by absolute or dilute alcohol. Gram's solution of iodine and potassium iodide causes no change of color either in the cold or after heating. No change of color occurs after treatment with iodine, heating, and addition of strong sulphuric acid. Dilute osmic acid blackens the granules instantly. Absolute alcohol used after osmic acid does not dissolve them; absolute alcohol and ether, after treatment with osmic acid, dissolves the granules.

From these tests it seems to be clear that the granules are fatty in their nature. The animals of depressed or starving cultures sometimes have few or none of these granules, while in vigorous cultures all the Euplotes have very numerous and well-developed ones. It appears then to be a fair conclusion that these peculiarly arranged granules of the ectosarc constitute a supply of reserve fatty food-substance.

Endosarc.—The endosarc presents a most irregular structure, differing according to the condition of the animal, whether full fed or starved, and the nature of the food. It is well to remember that a large part of what is commonly called endosarc is a mechanical mixture of protoplasm, food in various stages of digestion, water, and indigestible particles or excreta. In addition to the finely granular protoplasm which sections always show, the endosarc of this Euplotes contains great numbers of fibers. These are visible when the protoplasm has been crushed and torn during the process of sectioning and mounting. The constant motion of the central portion of the endosarc prevents there being any definite visible structural relation here.

The animals of well-fed cultures usually show large numbers of rounded, ovoid, or spherical granules, which are highly refringent, and form a conspicuous feature of the living endosarc. These spherules are unchanged by osmic acid, and are not dissolved by alcohol or ether, nor by the two together, but they are dissolved instantly by very weak solutions of nitric and hydrochloric acids and by strong acetic acid. They appear, therefore, to be a sort of calcareous concretion, bearing a certain resemblance to the crystals of calcium orthophosphate found in the endosarc of Paramœcium.

Ingestion and egestion.—The formation of a ball of food and its passage into the protoplasm is very much the same in Euplotes as in such a form as Paramœcium, except that there is no visible layer of water surrounding the food; no *food-vacuole* can be observed inside the body. The food mass appears to be in direct contact with the protoplasm, and while undoubtedly some water is swallowed with the food, there is not enough of it to be visible.

After entering the body the food mass is caught up by the circulating stream of protoplasm, which carries it forward, and then in a circle, counter clock-wise, for numerous small revolutions about the central region of the body. The cyclosis is continual but irregular in rate, and much more restricted in extent than in most large ciliates. The endosarc of the edges and anterior and posterior extremities of the body seems to be very stable.

The place of egestion is immediately behind the outermost anal cirrus, on the ventral surface. Frequently large quantities of indigestible material accumulate in the body before egestion takes place. Then the balls of ejecta are pushed out with considerable force, at intervals of a few seconds, until all is disposed of. The slowness with which the masses of ejecta are at first pushed through the surface of the body, until the largest part passes the pellicle, and the suddenness and force with which they then are shot away, indicate that the pellicle around the anus is highly elastic and that the anus is kept closed by its contraction. It appears probable that in those ciliata which possess a definite anal spot, there is also a real canal in the protoplasm, ordinarily kept closed and invisible by the elasticity of its walls and the pellicle.

As the anus lies just in front of the contractile vacuole, the masses of ejecta often press against its walls. In some cases the masses of undigested material accumulate to such an extent as actually to be forced into the contractile vacuole and cause it to become enormously distended; but in most cases this organ is only crowded to one side and partially surrounds the mass of waste material, the appearance that ejecta are inside the vacuole being deceptive.

Contractile vacuole.—The position of this organ is shown by Plate I, figure 1. When fully formed the vacuole is rather large. The pulsations occur at unusually long intervals. Because of the slowness of the process it is difficult to watch the entire cycle in an animal active enough to make the observer sure that it is in a normal condition. I find that the time required for the complete cycle averages twelve minutes. If, as has been suggested, a principal function of the vacuole is to force out of the body water which is continually being taken in with the food particles, the long period of the vacuole of Euplotes may very well be due to the extremely minute quantities of water absorbed with the food. The length of time required for a cycle of the contractile vacuole in this species is the more remarkable since Rossbach gives 31 seconds as the period of *Euplotes charon* O. F. M., and Maupas 50 seconds as that of *E. patella* O. F. M. I find no statement concerning the contractile vacuole of *E. vannus* O. F. M. in the summary of Minkiewicz's paper. It is possible that in this genus we have an opportunity to test the theory of a relation between the amount of water swallowed with the food and the activity of the contractile vacuole. Another indication of the low activity of the contractile vacuole of this species is in the fact that at any time large numbers of healthy animals show no vacuole at all. Out of twenty which were carefully examined at one time, the examination of each requiring about one minute, thirteen showed no vacuole, while in some of the remaining seven which had vacuoles, these were very small. In a less careful examination the contractile vacuole was visible in only four out of fifty, but I afterwards found that the vacuole is so minute for some minutes as to be nearly invisible even with high powers.

The contractile vacuole in Euplotidæ is said to open upon the ventral surface of the body beside or in front of the anus, but I have not been able to make any observations regarding this point, except to be certain that it does not open in front of the anus in *E. worcesteri.*

Nuclei.—The shape of the meganucleus is shown by text figure 10. The enlargement of the posterior end, reminding one of an anchor-fluke, is a most characteristic feature of this species of Euplotes. While it is subject to variation, so that the enlargement is not of exactly the same size nor shape in any two specimens, the general form is almost always the same, and so marked as to constititute one of the diagnostic characters of the species. Minkiewicz represents (text figure 13) the corresponding end of the meganucleus of *E. vannus* O. F. M. as turned back upon itself, but not enlarged. It does not seem possible that there could be a mistake in so simple an observation. The difference in the shape of this part of the meganucleus forms a very convenient means of distinguishing the two species, which otherwise are much alike.

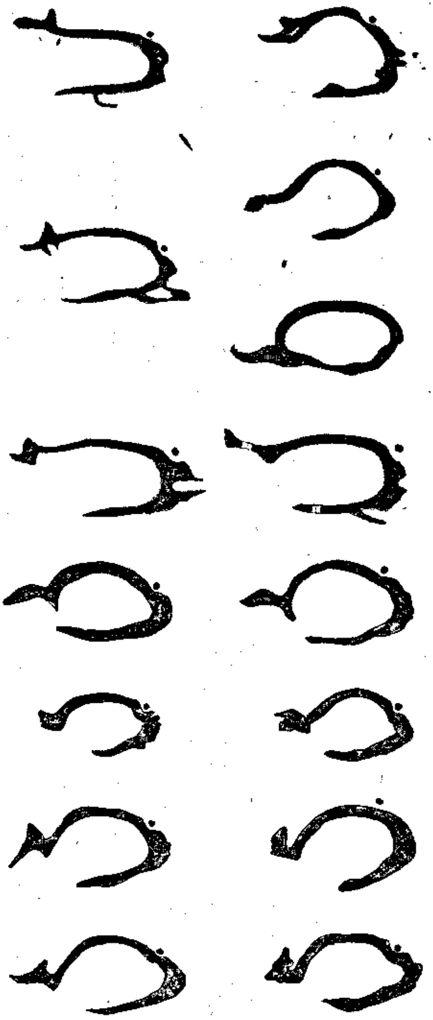

FIG. 10.—Various shapes assumed by the meganucleus of *Euplotes worcesteri* n. sp.

The portion of the nucleus which crosses the anterior end of the body is also considerably thickened and the anterior margin is frequently irregular.

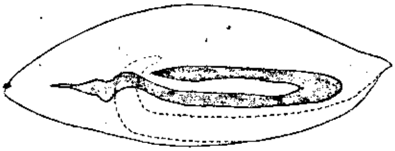

FIG. 11.—Lateral view of *Euplotes worcesteri* showing the curvature of the meganucleus over the pharynx.

The meganucleus lies close to the ventral surface of the body. In order to pass the pharynx, it bends over the dorsal side of this organ, as shown in text figure 11.

The chromatin of the meganucleus is arranged in a fine reticulum, as is shown by Plate IV, figures 1, 2, 3, and 4. It is generally found that the fibers of the reticulum on the anterior side of the anterior enlargement of the meganucleus are greatly attenuated, causing that part to appear less deeply stained than others. The nucleus is rarely visible in living animals. If the Euplotes are in a very clear, transparent condition, the meganucleus can sometimes be seen and its structure examined with an immersion lens. Under these circumstances the granular reticulation and clear, fluid ground-substance can be seen, the structure appearing the same as in the stained specimens.

At times when division is common and the animals seem to be well nourished and normal in every way, the interior of the nucleus of many animals becomes vacuolated. The vacuoles increase in size until all the protoplasmic portion of the nucleus is crowded into a layer along the nuclear membrane, while the interior consists of one or several large vesicles of clear fluid. It is only when in this condition that the nucleus is easily visible in the living animal. As the vacuoles increase in size

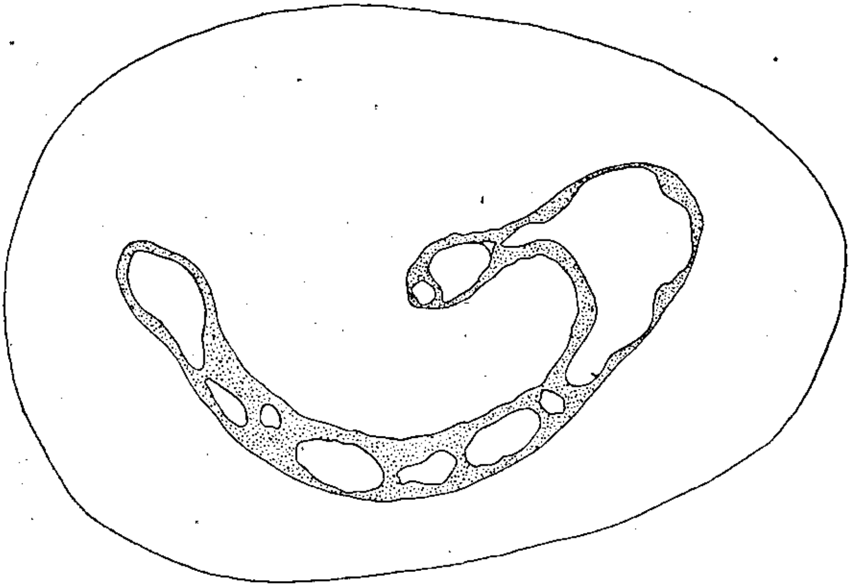

FIG. 12.—Sketch of a vacuolated meganucleus, drawn from the living animal.

the nucleus shortens and becomes thicker. The thickening may take place only around the vacuoles, while the intervening portions become drawn out into fine connecting threads, which finally break. Division may begin during this vacuolated condition of the meganucleus, but apparently does not proceed to a normal conclusion.

The micronucleus (Plate III, figure 9) is single, lying in or near a shallow depression on the left side of the meganucleus. I believe that the forms possessing two micronuclei, which are very common at times, belong to a stage following conjugation.

The micronucleus contains a sparse reticulum of chromatin. (Plate IV, figures 1, 2, 3.) Ordinarily the entire micronucleus stains uniformly, but with careful extraction of the stain the reticulum is rendered visible. If extraction is not quite sufficient, the micronucleus appears homogeneous, as if composed of a uniformly staining colloid substance.

Systematic position.—In order to distinguish *Euplotes worcesteri* from *Euplotes vannus* O. F. M., one must depend largely upon the careful work of Minkiewicz for exact knowledge of the structure of the European form. In the shape of the body, and in the number, form, and arrangement of the cirri, the Philippine form is exactly like the European.

Euplotes worcesteri has a longer pharynx than *E. vannus* O. F. M. and twenty to thirty membranellæ inside the pharynx instead of eight. Both the length of the pharynx and the number of pharyngeal membranellæ vary, but I have always found the number of these membranellæ to be more than double that given for *E. vannus* by Minkiewicz.

Minkiewicz presents one figure showing ten rows of sensory bristles on the dorsal surface of *E. vannus*. I find that most specimens of *E. worcesteri* have eight rows, although six, seven, nine, or ten rows are sometimes found. As there may be considerable variation in the number of these rows in *E. vannus* also, we are not justified in considering the apparent difference between the species in this regard as a valid specific distinction.

The most important difference, and the one upon which the diagnosis of the species really depends is in the shape of the posterior end of the meganucleus. As may be seen by referring to text figure 13 this end of the nucleus is enlarged in a very characteristic manner. Minkiewicz gives six figures of the meganucleus, all of which represent its end as being turned back upon itself, without any enlargement. (Text figure 13.) The general appearance produced is much the same as is seen in *E. worcesteri*, but the structure is entirely different.

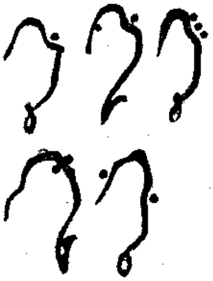

FIG. 13.—Drawings of the meganuclei of *Euplotes vannus* O. F. M., copied from Minkiewicz (Plate II, figure 23).

Whether differences exist in the arrangement of the ventral sensory bristles can not be said until *E. vannus* has been reëxamined with especial regard to these structures. The same is true of the suboral membranellæ and rows of endoral cilia.

The principal specific characters of *Euplotes worcesteri* are the following: Body oval, anterior end rounded, posterior end bluntly pointed; dorsal surface considerably elevated, with a regular curvature; anterior half of right margin usually straight; body 72 μ to 93 μ in length, 47 μ to 60 μ in width. Ventral surface flat; peristome considerably excavated and broad, extending two-thirds the length of the body, containing 45 to 70 wide membranellæ; mouth irregularly oval, large; pharynx long, recurved, containing 20 to 30 membranellæ, several oblique rows of endoral cilia, and a group of suboral membranellæ; 7 frontal cirri, 3 abdominal cirri, 5 anal cirri, 2 left marginal cirri, 0–3 right marginal or caudal cirri; sensory bristles of the dorsal surface usually in eight longitudinal rows; on the ventral surface paroral, exoral, and lateral rows of sensory bristles, and two to four bristles at the base of each cirrus. Meganucleus long, horseshoe-shaped, with a fluke-like enlargement at the posterior end and a considerable thickening where it bends across the anterior end of the body. Micronucleus single, lying in a slight depression of the meganucleus, on the left side near the anterior end of the body.

The dimensions of ten specimens of *E. worcesteri*, selected at random, are:

Specimen No.	Dimensions.
	Microns.
1	76×47
2	85×60
3	93×60
4	72×47
5	85×56
6	76×47
7	89×60
8	85×60
9	81×56
10	76×51
Average size	82×54
Least size	72×47
Greatest size	93×60

ILLUSTRATIONS.

PLATE I.

Ventral view of *Euplotes worcesteri* sp. nov. Numerous refringent endosarc spherules are visible, as well as three food-masses.

PLATE II.

FIG. 2. View of *Euplotes worcesteri* from the left side.

3. The dorsal surface of *Euplotes worcesteri* showing the sensory bristles and radiating fatty granules.

4. The ventral surface of *Euplotes worcesteri* showing the positions of the sensory bristles, the radiating fatty granules, and the contractile fibrils running to the bases of the cirri.

PLATE III.

FIG. 5. A longitudinal section of *Euplotes worcesteri.* Camera lucida drawing; Zeiss compensation-ocular 6 and obj. $\frac{1}{12}$. At the anterior end the slight overhang of the dorsal surface and the marginal lamella are shown. The section passes just mediad to the inner wall of the persistome and mouth, cutting the pharynx, in which are seen the pharyngeal and suboral membranellæ. The meganucleus is cut in two places. The smaller black bodies are nuclei of small flagellates eaten by the Euplotes. The grouping of food-bodies is characteristic.

6. A portion of the ectosarc in vertical section. Within the ectosarc alveoli is a portion of endosarc, and a large food-body. The contents of the ectosarc alveoli usually do not stain. Camera lucida drawing.

7. A tangential section of the ectosarc. Camera lucida drawing.

8. A section through one of the anal cirri, its basal plate, and its contractile fibril. The cilia of the cirrus are in the usual twisted condition. Camera lucida drawing.

9. A micronucleus, stained with iron-hæmatoxylin; stain almost completely extracted, bringing into view the faint chromatin reticulum.

TEXT FIGURES.

FIG. 1. Outline sketches of three of the deformed individuals which appear when cultures have passed their prime. Specimen A seems to be the result of an aborted division.

2. A cross section passing through the mouth, the adoral membranellæ, *a. z.*, and the suboral membranellæ, *s. o.*

3. Cross section passing through the mouth, the suboral membranellæ, the pharynx and pharyngeal membranellæ, and the adoral zone.

4. A longitudinal section, showing the antero-ventral groove of the pharynx.

5. A tangential section passing through the dorsal wall of the pharynx. The rows of basal granules of the pharyngeal membranellæ and of the endoral cilia show the arrangement of these organs.

FIG. 6. Part of a longitudinal section passing just inside the mouth. *s. o.*, suboral membranellæ; *e. o.*, endoral cilia, which in this section are well down on the anterior wall.

7. Basal granules of three adoral membranellæ and the outlines of the grooves about their bases.

8. Camera lucida sketch of a tangential section of the ventral surface, showing the rows of basal granules and some of the contractile fibrils of the cirri.

9. Contractile fibrils of the cirri, drawn from an unstained specimen fixed in corrosive-formol-acetic, followed by alcohol.

10. Various shapes assumed by the meganucleus of *Euplotes worcesteri.*

11. Lateral view of *Euplotes worcesteri* showing the curvature of the meganucleus over the pharynx.

12. Sketch of a vaculated meganucleus; drawn from the living animal.

13. Drawings of the meganucleus of *Euplotes vannus* O. F. M., copied from Minkiewicz. (Plate II, figure 23.)

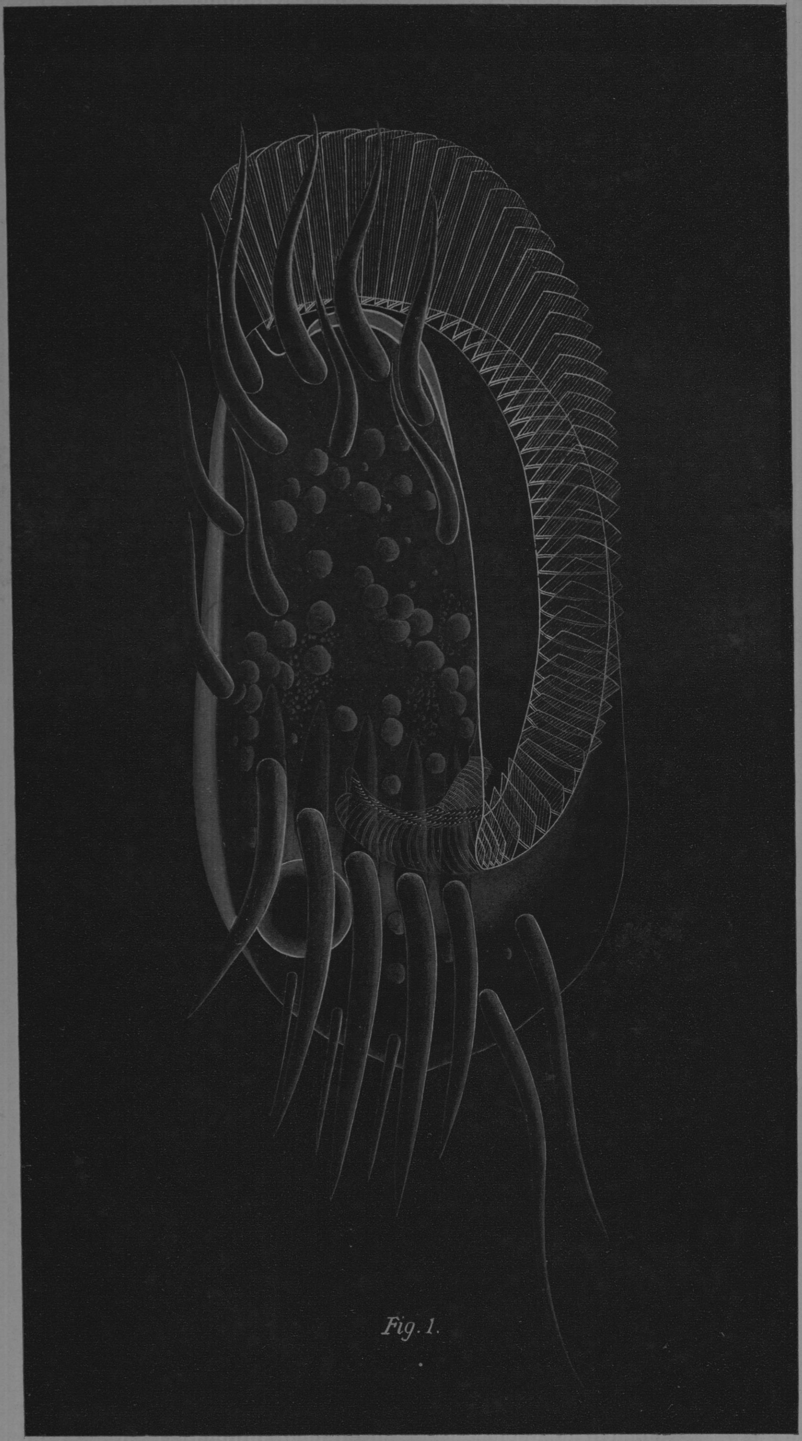

Fig. 1.

L. E. Griffin and T. Espinosa.

PLATE I.

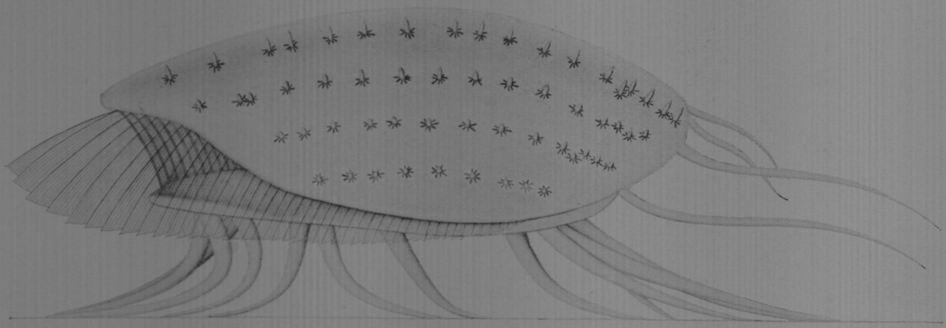

Fig. 2.

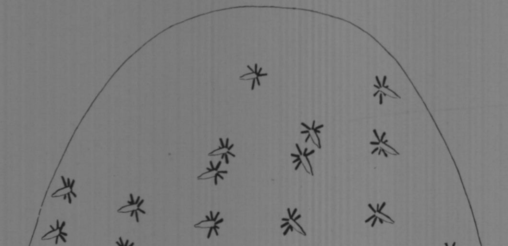

Fig. 3

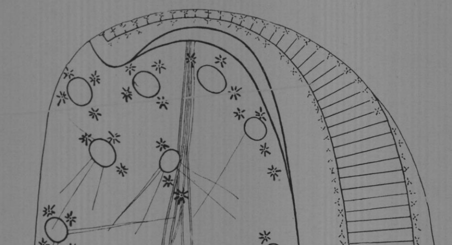

Fig. 4.

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PLATE II.

Fig. 5.

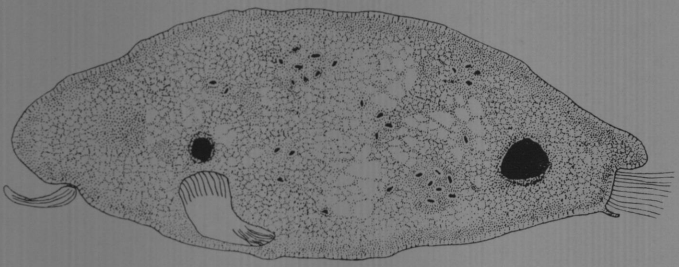


Fig. 6.

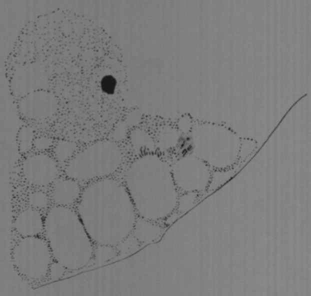
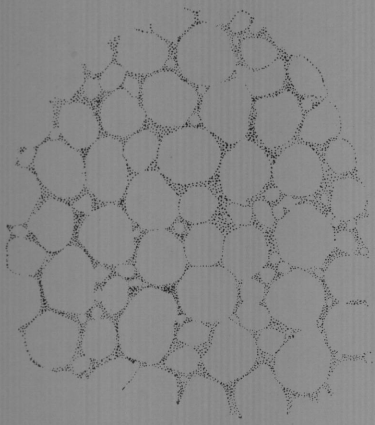


Fig. 7.



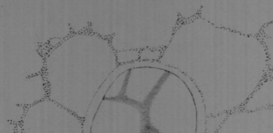


Fig. 9.

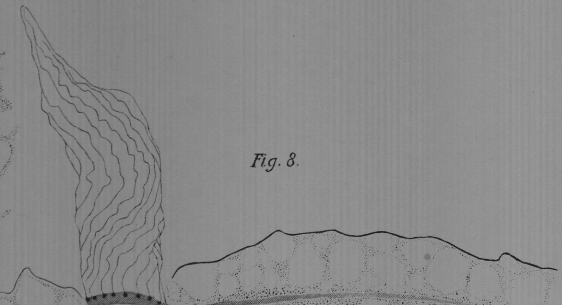


Fig. 8.

L. E. Griffin and T. Espinosa.

PLATE III.

REVIEW.

Check-list of North American Birds. Prepared by a Committee of the American Ornithologists' Union. 3d ed. revised. Cloth. Pp. 430. Price $3.50 net. New York: American Ornithologists' Union. 1910.

This welcome and long-promised volume is essentially the second edition of the check-list with the interpolation of the various additions and other changes which have been recorded in the supplements since 1895. The details of geographical distribution, *"range"* in the new check-list, are given in much greater detail than ever before, especial attention having been devoted to defining the breeding and seasonal ranges of species and subspecies. The scientific name and vernacular name of each species, or subspecies, are printed on the same line, followed by the old check-list number. The names of authorities are spelled out. The secondary references and concordance to previous check-lists are omitted, but type localities are given. Names of subspecies are printed in slightly smaller type than those of species and are designated *a, b, c,* etc. This is perhaps an advantage so long as trinomials are retained, but the reviewer has not discovered the advantages of trinomials. Vernacular names seem to have received but limited attention, the treatment of double names especially being most unhappy. Thus we find Water-Turkey, Wood Duck, Tree-duck, Tree Sparrow, Pintail, Spoonbill, Red-head, Buffle-head, Canvas-back, Tropic-bird, Surf-bird, Catbird, Cowbird.

Two maps, one colored, show the life zones of North America and the localities mentioned in the check-list.

The paper, press work, and binding of the new check-list are very similar to those of the second edition, that is to say, excellent.

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